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Gastroprotective Effect of the Three Glucuronopyranoside Flavonoids in Rats

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In this study, we investigated the protective action of glucuronopyranoside flavonoids (QGC, AGC, LGC) on gastritis in rats. QGC, AGC and omeprazole decreased the gastric volume significantly, and each ID_{50} was 0.75, 0.54 and 8.5 mg/kg, respectively, thus the order of potency was AGC, QGC and omeprazole. They also decreased acid output, and each ID_{50} was 7.81, 0.58 and 6.71 mg/kg, respectively, thus the order of potency was AGC, omeprazole and QGC. They inhibited gastritis induced by indomethacin, and it recovered significantly by increasing the GSH levels in gastritis. The gastric MPO activity in the gastritis group increased more than in the normal group. QGC, LGC, or AGC administration reduced moderately the MPO activity in a dose-dependent manner. This study demonstrated that AGC, QGC, or LGC showed potent efficacy on the gastritis, by preventing oxidative stress. These results suggest that QGC, AGC, or LGC have gastroprotective effect in rats.

Key Words: Flavonoids, Gastritis, Lipid peroxidation

INTRODUCTION

Reflux esophagitis is a common disease entity in which the gastric juice gains access to the esophagus via transient lower esophageal sphincter (LES) relaxation [1-8], speed of esophageal clearance, mucosal resistance and other factors, and is often associated with a low pressure in the LES [9]. Gastric acid, pepsin and bile irritate the squamous epithelium, leading to erosion and ulceration of esophageal mucosa. Eventually, a columnar epithelial lining may develop and if untreated, may result in chronic esophagitis, aspiration pneumonia, esophageal strictures and Barrett's esophagus, a premalignant condition [10].

Oxygen derived free radicals have been known to play an important part in the pathogenesis of the injury of various tissues including the digestive system [11], and lead to acute gastric and esophageal mucosal injury as a result ischemia [12,13], or ethanol [13]. Gastritis involve inflammatory changes in the gastric mucosa, including erosion caused by a noxious irritant from exposure to bile and pancreatic fluids, hemorrhagic gastritis, infectious gastritis, and gastric mucosal atrophy. ROS generate lipid peroxidation which is believed to be an important cause of destruction and damage of the cellular membranes.

In the current our study the involvement of extensive lipid peroxidation in ethanol-induced gastric damage was evidenced by the accumulation of MDA level, as an index of lipid peroxidation, and MPO activity, as a marker of neutrophil aggregation [14]. It has been demonstrated that free radical damage to the gastric or esophageal mucosa can be prevented by the administration of free radical scavengers [15].

We have shown that glucuronopyranoside flavonoids had inhibitory effect for reflux esophagitis and gastritis [14-17]. H2-receptor antagonists and prokinetic agents promote symptomatic relief and esophageal healing in mild esophagitis, but are less effective for the treatment of moderate to severe esophagitis.

The goal of this study was to evaluate the gastroprotective effect of three flavonoids containing glucuronopyranoside in rats, and also compared GSH or MPO levels to determine gastroprotective and antioxidative effects.

METHODS

Gastric acid secretion in reflux esophagitis

Male Sprague-Dawley rats with a body weight of about 200 g were used for the experiments. The rats were housed in a controlled room (temperature of 22±20°C, relative humidity of 50±5%) with a defined light/dark cycle and were

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ABBREVIATIONS: QGC, quercetin-3-O-β-D-glucuronopyranoside; AQC, apigenin-7-O-β-D-glucuropyranoside; LQC, luteolin-7-O-β-D-glucuropyranoside; PI-LPC, phosphatidyl choline-phospholipase C; MPO, myeloperoxidase; GSH, glutathione sulfhydryl; ROS, reactive oxygen species; SOD, superoxide dismutase; CAT, catalase.

given a solid diet and tap water *ad libitum*. All animals were fasted over night before experiment.

All experiments were approved by the Institutional Animal Care and Use Committee of Chung-Ang University, in accordance with the guide for the Care and Use of Laboratory Animals in Seoul, Korea. Under ether anesthesia, the abdomen was incised along the midline and then both the pylorus and limiting ridge (transitional region between the forestomach and corpus) were simultaneously ligated [1]. A longitudinal cardiomyotomy of about 1 cm in length across the gastroesophageal junction was performed to enhance reflux. Six hours later, the animals were sacrificed by cervical dislocation and then the esophagus was harvested.

Six hours after pylorus ligation, rats were sacrificed by cervical dislocation and the esophagus clamped, as previously confirmed reflex esophagitis [15]. Samples of gastric juice were collected in graduated conical centrifuge tubes and centrifuged at 3,000 g for 10 min at 4°C. After centrifugation, the volume (ml/rat) and acidity (mEq/l) of the supernatant was measured. Total acidity was determined by titration of the gastric juice against 0.1 N NaOH to pH 7.0. Acid output was expressed as μEq/hr [5]. Each ID_{50} was calculated that indicate 50 percentage inhibiting dose on gastric volume and acid output, respectively.

Induction of indomethacin-induced gastritis

Six hours after oral (50 mg/kg) administration of indomethacin [6], the rats were sacrificed, and then their stomachs were excised. The stomachs were opened along the greater curvature and spread out with pins on a corkboard. The sum of length (cm) of the mucosal erosive lesions was measured under a dissecting microscope using a squared grid (X10; Olympus, Tokyo, Japan).

QGC, AGC, LGC, or omeprazole were administered *per os* (p.o.) one hour before indomethacin administration. The volume of the drug or vehicle was 2 ml/kg of body weight. The agents were prepared freshly each time.

GSH assay

GSH concentration of stomach mucosa tissue was determined according to Beutler method [18] and a partially modified method was used. The obtained mitochondrial fraction was added to metaphosphoric acid for protein precipitation and stand for 5 min. Phosphate buffer and 5′5-dithiobis-2-nitro-benzoic acid were then added for color development. GSH was determined by spectrophotometrically at 415 nm using GSH.

Measurements of MPO assay

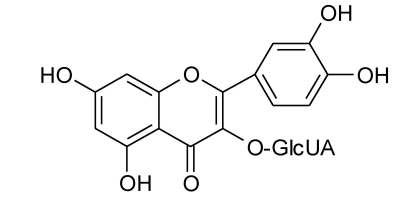
The MPO assay was based on the method of Grisham [19] and partly modified. One milliliter of leukocyte suspension of the stomach in the rats was centrifuged at 620× g at 4°C for 2 min. The precipitate was suspended in 1 ml of 80 mM sodium phosphate buffer, pH 5.4, containing 0.5% hexadecyltrimethlyammonium bromide (0.5% HETAB solution), freeze-thawed 3 times and centrifuged at 1,400×g at 4°C for 5 min. Duplicate 30 μl samples of resulting supernatant were poured into 96 well microtiter plates. For assay, 200 μl of a mixture containing 100 μl phosphate buffered saline, 85 μl 0.22 M sodium phosphate buffer, pH 5.4, and 15 μl of 0.017% hydrogen peroxide were added to the wells. The reaction was started by the addition of 20 μl of 18.4 mM TMB · HCl in 8% aqueous dimethylformamide. Plates were stirred and incubated at 37°C for 3 min and then placed on ice where the reaction was stopped by addition to each well of 30 μl of 1.46 M sodium acetate, pH 3.0. The MPO value was evaluated by measuring the absorbance of samples at 620 nm (OD value) and being converted it into MPO value.

Agents

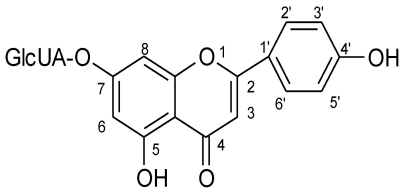
AGC, QGC and LGC (Fig. 1) were obtained from *Clerodendron trichotomum, Rumex Aquaticus, Salix gilgiana*, respectively, where each was seperated and extracted [15-17]. Thiobarbituric acid, trichloroacetic acid, malonaldehyde bis (dimethyl acetal), bovine serum albumin, *o*-phthalaldehyde, diethylene-triaminepentaacetic acid, ascorbic acid, *N*-ethylmaleimide, omeprazole and glutathione were purchased from Sigma (St. Louis, MO, USA). Potassium phosphate were purchased from Showa (Tokyo, Japan). Protein assay kits were purchased from BioRad (Richmond, CA, USA).

Analysis of data

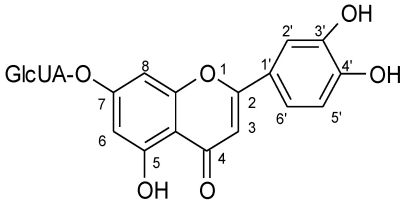
Data are expressed as means±SEM (standard error of means). Statistical differences between means were determined using the Student's *t*-test. A p value below than 0.05 was considered as statistically significant.



QGC: quercetin-3-O-β-D-glucuronopyranoside



AGC: apigenin-O-7-β-D-glucuronopyranoside



LGC: luteolin-O-7-β-D-glucuronopyranoside

Fig. 1. Chemical structures of QGC, AGC and LGC.

RESULTS

The comparison of QGC, LGC, AGC, and omeprazole on gastric volume and acid output in reflux esophagitis

It was confirmed the inhibition of reflux esophagitis by treatment of these 3 compounds. Thus QGC, AGC and omeprazole decreased the gastric volume significantly in a dose dependent manner, and each ID_{50} was 0.75, 0.54 and 8.5 mg/kg, respectively, thus the order of potency was AGC, QGC and omeprazole. However, ID_{50} of LGC was larger than 30 mg/kg (Table 1). They significantly decreased acid output and the ulceration of gastric and esophageal mucosa, as was observed for the proton pump inhibitor, omeprazole. Each ID_{50} was 7.81, 0.58 and 6.71 mg/kg, respectively, thus the order of potency was AGC, omeprazole and QGC. However, ID_{50} of LGC was larger than 30 mg/kg.

The inhibition of AGC, LGC, QGC in indomethacin-induced gastritis

Six hours after oral (50 mg/kg) administration of indomethacin, the stomachs of rats were opened, and the sum of length (cm) of the mucosal erosive lesions was measured QGC, LGC, AGC or omeprazole was decreased gastric ulcer lesion, in a dose dependent manner. LGC had the highest inhibitory activity among the compounds tested (Fig. 2). The order of inhibitory potency was LGC, AGC, QGC and omeprazole.

Table 1. The ID_{50} comparison of gastric volume and acid output in esophagitis

Agents	ID_{50} (mg/kg, PO)	
	Gastric volume	Acid output
Omeprazole	8.50±1.90	6.71±0.89
AGC	0.54±0.21	0.58±0.15
LGC	>30	>30
QGC	0.75±0.29	7.81±0.91

QGC, LGC and AGC decreased the gastric volume and acid output in a dose dependent manner. It was calculated ID_{50} indicated the 50% inhibiting dose, respectively. Data are expressed as mean±SEM of 6~7 animals.

The recovering effect of QGC, LGC, AGC on glutathione levels in gastritis

As shown in Fig. 3, in the indomethacin-induced gastritis model, the glutathione levels were significantly lower than the level of normal group, respectively ($p<0.01$). QGC, LGC, or AGC administration (0.1, 0.1, 1, 3, or 10 mg/kg) recovered significantly by increasing the GSH levels. The order of recovering potency was QGC, LGC, AGC and omeprazole, respectively. QGC was most affected. Omerpazole used as control, and it had no effect on glutathione levels, but only affected at high dose (10 mg/kg).

Inhibitory effects of QGC, LGC, AGC and omeprazole on myeloperoxidase levels in gastritis

In the gastritis induced by indomethacin, the gastric MPO activity significantly increased 3.6 times more than in the normal group (Fig. 4). QGC, LGC, or AGC administration reduced moderately the MPO activity in a dose-dependent manner. The order of recovering potency was QGC, AGC, LGC, respectively. QGC was most affected. Omerpazole used as control, and it had no effect on myeloperoxidase levels, but only affected at high dose (10 mg/kg).

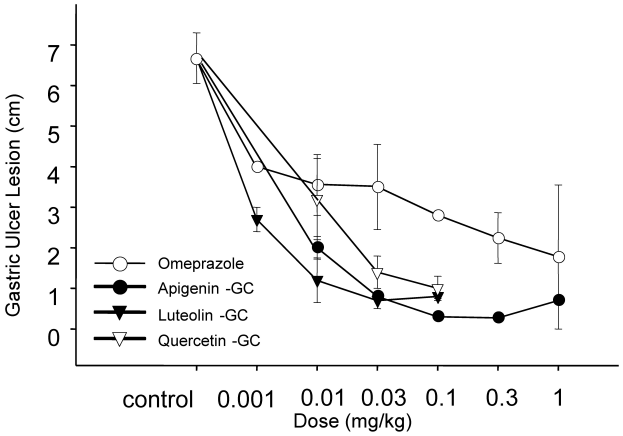


Fig. 2. The effect of QGC, LGC, AGC and omeprazole on gastritis. Data are expressed as mean±SEM.

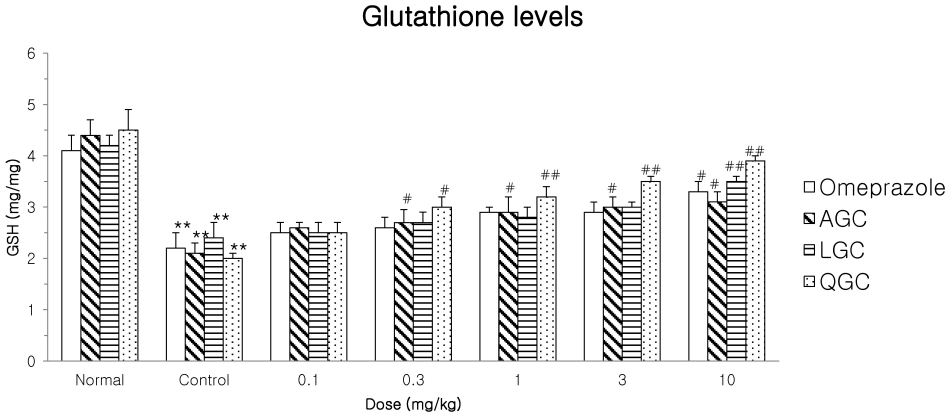


Fig. 3. The effect of QGC, LGC, AGC and omeprazole on glutathione levels in gastritis. In the control gastritis group, the glutathione levels were significantly lower than the levels in the normal group. However, QGC, LGC, or AGC administration recovered the GSH levels. Data are expressed as means±S.E.M, n=6~8. **$p<0.01$ compared with each normal value. $^{\#}p<0.05$, $^{\#\#}p<0.01$ compared with each control group.

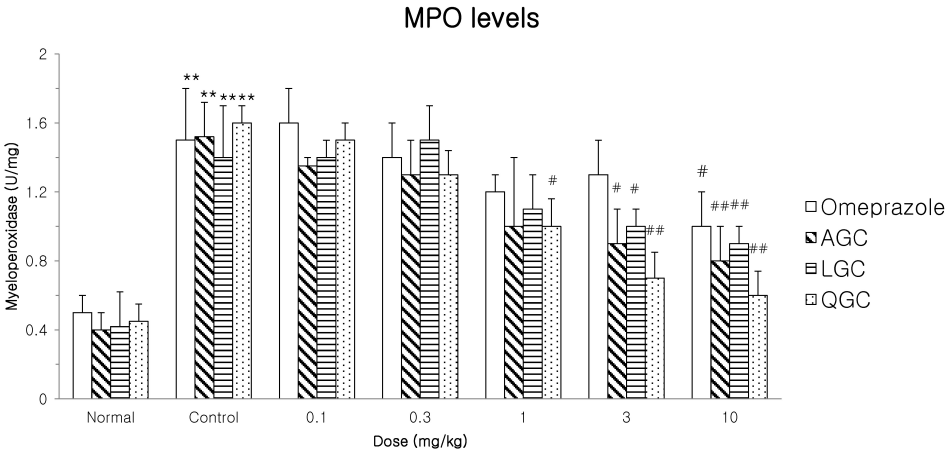


Fig. 4. Inhibitory effects of QGC, LGC, AGC and omeprazole on myeloperoxidase levels in gastritis. In the gastritis group, the gastric MPO activity significantly increased than the normal group. QGC, LGC, or AGC administration reduced the MPO activity in a dose-dependent manner. Data are expressed as means± S.E.M, n=7. $^{**}p<0.01$ compared with each normal value. $^{\#}p<0.05$, $^{\#\#}p<0.01$ compared with each control group.

DISCUSSION

QGC, AGC and LGC inhibited the gastric acid output and had more potent efficacy than omeprazole in regards to inhibiting acid output. It has been reported that acid output inhibition greater than 40% is enough to prevent the development of esophagitis [5] and gastric acid is considered essential to esophageal mucosal damage [20].

Present experiments demonstrated that these compounds showed potent efficacy on the development of reflux esophagitis and indomethacin-induced gastritis, by the inhibition of gastric secretion and the prevention of oxidative stress. Our results demonstrated that these compounds display antiulcer, gastroprotective, gastric antisecretory activities, as observed for omeprazole. Flavonoids also have gastric antisecretory activity [3,6,20]. It has been reported that the pH-dependent pattern of mucosal injury is best explained by the very low activity of gastric pepsin over pH 4.0 [21]. It has also been reported that the effect of antisecretory therapy on reflux esophagitis can be predicted from the duration of suppression of intragastric acidity above pH 4.0 achieved by each drug regimen [20]. This activity occurs through different mechanisms. For example, it has been reported that flavonoid compounds inhibit gastric H^+, K^+-ATPase, where the inhibition is competitive with respect to ATP [22].

It has been reported that the gastric antisecretory activity is as effective as cimetidine in reducing gastric acid secretion [23]. Glucuronide flavonoids have antiulcer and gastroprotective activity. It was shown to have antiulcerogenic properties in rats and guinea pigs; such properties appeared to be of interest with respect to the adverse effect of gastric ulceration, which develops commonly in subjects taking anti-inflammatory drugs [24].

In our study, QGC, AGC and LGC inhibited indomethacin-induced gastritis. It is well known that induction of gastric lesions by indomethacin is the oxidative damage with its dual events of reactive oxygen species generation [25] and lipid peroxidation [26]. Indomethacin activates the polymorphonuclear leucocytes in peripheral blood and enhances the release of ROS from these cells [27]. ROS generate lipid peroxidation which is believed to be an important cause of destruction and damage of the cellular membranes. In the current study the involvement of extensive lipid peroxidation in indomethacin-induced gastric damage was evidenced by the accumulation of MDA level, as an index of lipid peroxidation, and MPO activity, as a marker of neutrophil aggregation.

In experiments on glutathione, the concentration was decreased after indomethacin-induced gastritis, and GSH significantly recovered after 3 compounds administration. This results can support that the enzyme activity is reversibly inhibited by reactive oxygen species and oxidized glutathione [28].

From the findings in this study, the 3 compounds were proven significantly and dose-dependently to protect effectively against the NSAIDs-induced gastric damage, which was more susceptible to NSAIDs by the administration of indomethacin in rats. The underlying mechanism of gastroprotective effects of the gastric damage induced by indomethacin could be related to its anti-oxidant properties, which reduce MDA levels, MPO activity. In further experiments, it mains to be examined SOD, CAT activities and SOD-2 expression which levels may increase. Therefore, these results suggest that 3 flavonoids have the inhibitory action on the development of indomethacin induced gastric damage in rats.

We have shown that reduced GSH levels could be due to the oxidation of SH groups following ethanol-induced generation of ROS or due to the binding of SH groups by acetaldehyde generated from metabolism of ethanol by gastric alcohol dehydrogenase [14]. In accordance with these demonstrations, the gastritis model induced by indomethacin used in our study also exhibited the reduced GSH levels by the reduction of antioxidative enzyme activity, demonstrating that the gastritis may be induced by oxidative stress.

Administration of LGC, QGC, or AGC in rats significantly attenuated MPO levels increased by indomethacin. Their inhibitory activity may result from its antioxidant activity and its ability to scavenge ROS produced by indomethacin administration which initiated lipid peroxidation. In the present study, the increases in antioxidant levels can be explained by gastroprotective effect of those compounds through its antioxidative action. They prevented the increase of myeloperoxidase, indicating that gastric mucosa was protected from the deleterious effects of activated neutrophil infiltration and that they may have antiinflammatory properties in gastric mucosa.

This study showed that 3 compounds showed potent efficacy on indomethacin-induced gastritis in rats. These results suggest that QGC, AGC or LGC might be a promising

compound due to gastro protection by antioxidation.

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